# CHANGES IN ANATOMICAL AND FUNCTIONAL CONNECTIVITY RELATED TO LOWER HIPPOCAMPAL VOLUME

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**Target audience**: Scientists and clinicians interested in early diagnosis of cognitive impairments using brain connectivity analysis.
**Purpose:** To analyze structural and functional connectivity (SC-FC) differences which accompany structural changes in the hippocampus, in order to obtain early biomarkers for probable Alzheimer's disease (AD) development.
**Introduction:** Anatomical 3D T1 weighted images have been widely used to assess the volume of subcortical structures. It has been demonstrated that volume loss of hippocampi, entorhinal cortex and amygdala are early biomarkers for the diagnosis of cognitive impairments and AD[1]. In this work, we investigate AD biomarkers based on brain connectivity. SC-FC differences have been reported between controls and patients at risk for AD[2]. We will try to anticipate to MCI or AD diagnoses by differentiating in a sample of healthy subjects, i.e. with no cognitive impairment, other than related to ageing, using as criterion of separation the normalized hippocampal volume (NHV). The sample is formed by volunteers in the Valleca's Initiative, a longitudinal study evaluating normal ageing in a cohort of more than 600 healthy elder people (70-85 years). The prevalence of AD in people older than 65 years is 13%[3] suggesting that a certain number of those subjects will develop AD in the next years. The subjects with lower NHV are more prone to have AD than subjects with higher NHV. Thus they are more likely to manifest connectivity patterns that can be considered as AD biomarkers.
**Methods:** *Subjects:* 50 of 632 subjects (mean age 76±4years) were picked up for connectivity analysis. These 50 subjects form two groups according to the NHV, i.e. *hippocampal/gray matter volume* ratio. The first group included 25 subjects (16f / 9m) whose NHVs were standard deviations lower than the mean NHV, the second group included 25 subjects (16f / 9m) whose NHVs were one standard deviation above the mean NHV.
*Data acquisition:* The MRI acquisitions were performed on a GE Signa HDx 3T scanner. The MRI protocol consisted of high resolution anatomical 3D-T1-weighted image, TR/TE/TI = 10.024/4.56/600 ms, flip angle = 12º and voxel size = 0.83x0.83x1.00 $mm^3$; DWI in 21 directions ($b$ = 800 s/$mm^2$), TR/TE = 9200/86.7 ms, flip angle = 90º and voxel size = 3.60x3.60x3.00 mm; 5 minutes resting-state fMRI using a GE-EPI pulse sequence TR/TE = 2500/27.5 ms and voxel size = 2.5x2.5x2.6 mm.
*Data pre-processing*: T1-weighted images were processed with Freesurfer to obtain cortical (68) and subcortical (16) ROIs for each subject. These ROIs were registered to the DWI and fMRI spaces in order to define the nodes of SC-FC networks.
DWIs were corrected for motion, eddy currents and field inhomogeneities using FSL v5.0.5. fMRI was preprocessed for spiking, slice timing, motion, field inhomogeneities using FSL v5.0.5 and AFNI.
*Data post-processing*: Tractography analysis was performed using the deterministic tensor deflection tracking[4] implemented in Diffusion-Toolkit. SC is computed in two ways, as the track density between every pair of ROIs and as the track density normalized by the average volume of the seed nodes[5] in order to account for the different NHV between groups. FC was computed using Pearson and partial correlations between average time-series from the 84 ROIs.
Statistical analysis was performed to compare SC-FC networks between the two groups. We applied a Wilcoxon ranksum test and a Genovese FDR (q<0.01) to correct for multiple comparisons.

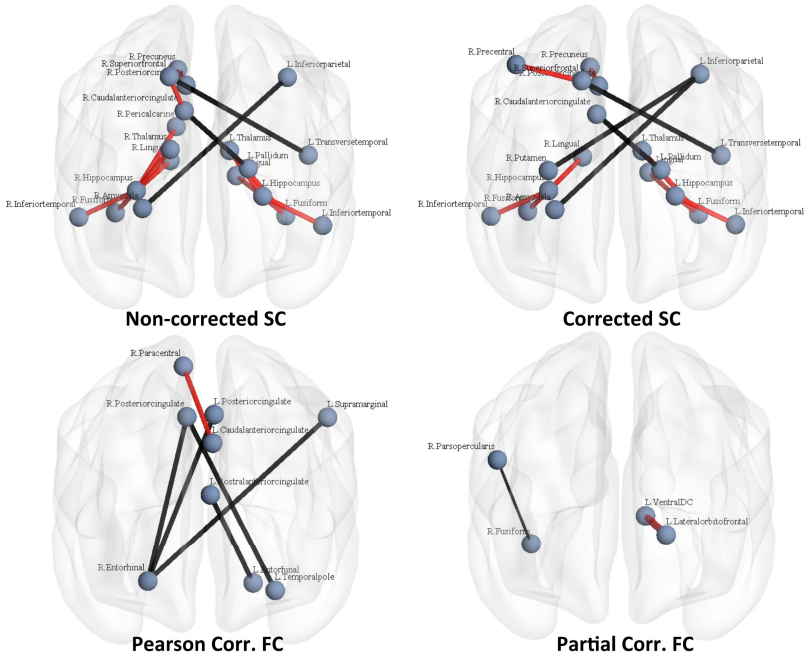


*Figure 1. First row shows coronal view of SC differences (FDR q<0.01) between groups. Second row shows coronal view of FC differences (uncorr. p<0.001) between groups. Red/black lines represent that connections are higher/lower in the group of higher NHV*

**Results:** Thirteen links survived the FDR for non-corrected SC and eleven in the case of corrected SC. Bilateral connections between hippocampi and fusiform/inferiortemporal/lingual cortices were higher in the higher NHV group. These connections also appeared when introducing volume correction for the SC. A higher SC was found between thalamus and fusiform cortex/hippocampi for the higher NHV group. We also identified significant higher connections in the lower NHV group including fronto-temporal and temporal-parietal connections, however these values range in an order of 10 to 100 times lower than other values of SC making their interpretation not straightforward. None of the FC differences between groups survived the FDR correction. We identified links with an uncorrected p-value of 0.001. A higher FC (Pearson correlation) was found in connections between the entorhinal cortices and the anterior and posterior cingulate. Only two connections showed differences between the groups with partial correlations FC including a frontal-thalamic connection.
**Discussion/Conclusion:** A SC decrease in subjects with a lower NHV was found in the temporal lobes. This decrease of connectivity is not only due to the NHV differences between groups, as the differences remained after volume correction. An increase in FC between entorhinal cortex and cingulate may represent a compensatory effect previous to the development of a cognitive impairment. Individuals at risk for AD show a decrease/increase of SC/FC, likely representing an upcoming neurodegeneration. We will test this hypothesis in the following years of this initiative.
**References**: 1. Dubois et al. *Lancet Neurol.* 2007;6(8):734–46. [2]. Wang et al. *Biol. Psychiatry*. 2013;73(5):472–81. [3]. *Alzheimer's Dement.* 2012;8(2). [4]. Lazar et al. *Hum. Brain Mapp.* 2003;18(4):306–21. [5]. Hagmann et al. *PLoS Biol.* 2008;6(7):e159
**Acknowledgements**: This work has been partially funded by project TEC2012-39095-C03-01 of the Spanish Ministry of Economy and Competiveness.